

Characterization of Allergens and Assessment of Immune Responses to *Stachybotrys Chartarum* and *Penicillium Chrysogenum*

YongJoo Chung ¹, Marsha D W Ward, Michael E Viana ², Lisa B Copeland, and MaryJane K Selgrade

¹SPH, University of North Carolina ² CVM, North Carolina State University

**SUSCEPTIBILITY,
ALLERGY & ASTHMA**

Introduction

The increased prevalence of allergic asthma and allergic disease among children in developed countries including the US has lead to research initiatives within EPA. Molds have been associated with allergic disease but have not been studied extensively despite of their widespread presence in the indoor environment. A cross-laboratory study was initiated to investigate children's health problems associated with indoor fungal exposure. Two fungi, *Stachybotrys chartarum* and *Penicillium chrysogenum*, under investigation have been associated with both water damaged buildings and sick building syndrome.

Objectives

1. to identify allergic (IgE inducing) proteins of *S. chartarum* and *P. chrysogenum*
2. to assess immune responses to *P. chrysogenum*
3. to evaluate the proinflammatory effect (potential adjuvant) of chrysolysin, a hemolytic component in *P. chrysogenum*.

Methods

Animals

- Fifty day-old BALB/c mice (Charles River Labs, Raleigh, NC)

Preparation for fungal extracts

- Five *S. chartarum* isolates (58-07, 58-16, 58-17, 58-18, and 63-01) or *P. chrysogenum* were ground with mortar and pestle, stirred overnight, clarified by centrifugation, filter sterilized, and concentrated to yield crude extracts, SCE and PCE, respectively.

1. Hyperimmune serum production

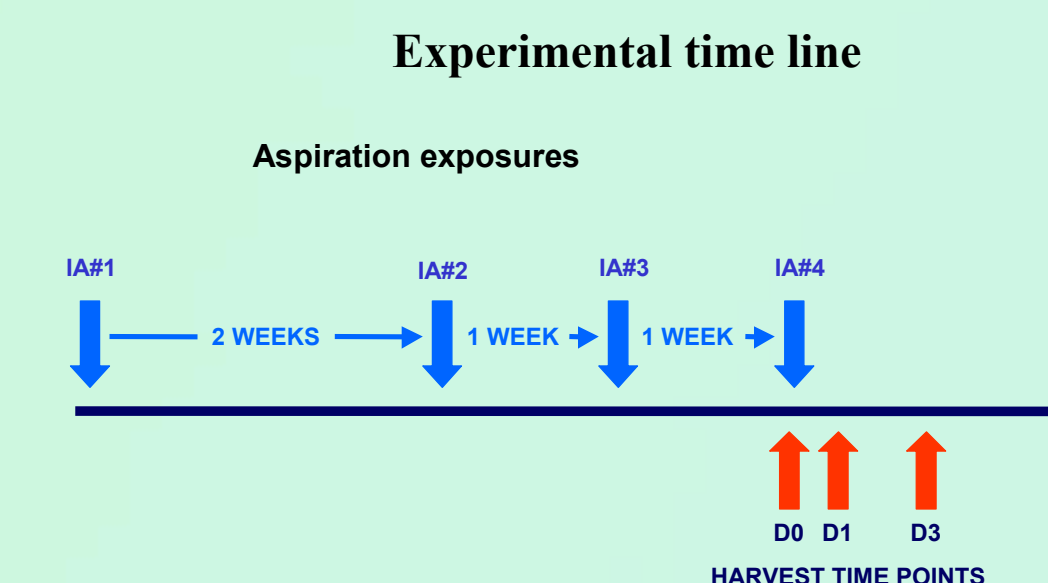
- 25 µg of SCE or PCE in 200 µl alhydrogel adjuvant were introduced to mice (i.p.)
- Three and six weeks later, 15 µg of extract in 200 µl HBSS were introduced to mice (i.p.)
- Mice were killed 5 days after the final injection by an overdose of sodium pentobarbital and exsanguinated by cardiac puncture.
- Blood was pooled, allowed to clot 1h at room temperature, spun down, and the resulting serum aliquoted and stored at -80°C.

Western blot analysis

- SCE components and PCE were separated by gel electrophoresis and electrophoretically transferred to PVDF membranes.
- The membranes were blocked with 5% non-fat dry milk [NFDM] in Tris-buffered saline + 0.1% (v/v) Tween-20 [TBS-T] for 1 hour at room temperature, incubated with hyperimmune serum at a 1:100-200 dilution in NFDM in TBS-T overnight at 4°C, then exposed to the secondary antibody at a 1:15000 ~ 20000 dilution in NFDM, and then visualized by enhanced chemiluminescence (Perkin Elmer).
- Relative mobilities were calculated for molecular weight standards run on all blots, and the approximate molecular weight of the antigen bands were calculated from a standard curve based on the relative mobility of the standards vs. the log₁₀ of the molecular weights.

2. Treatment for PCE-induced immune response

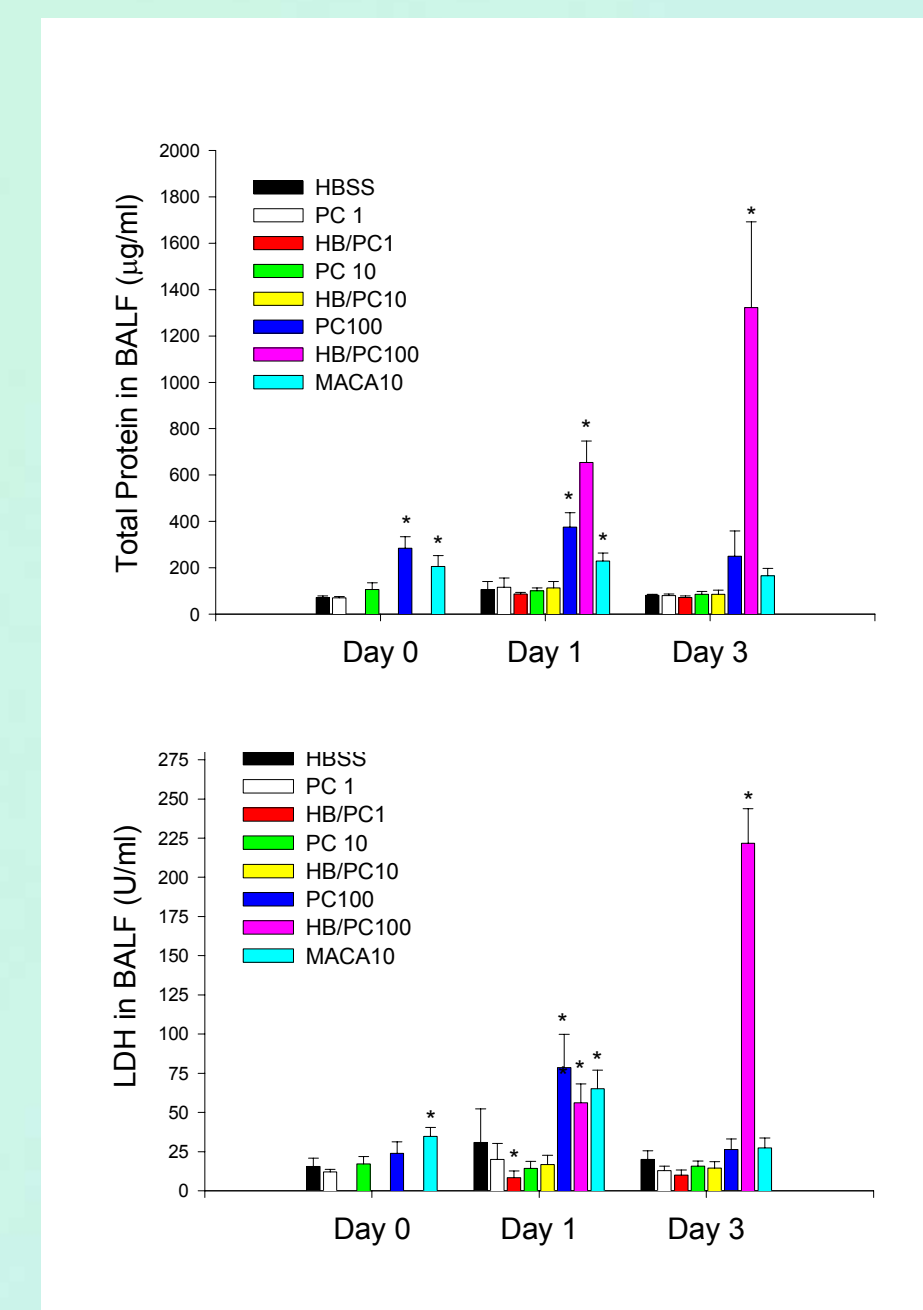
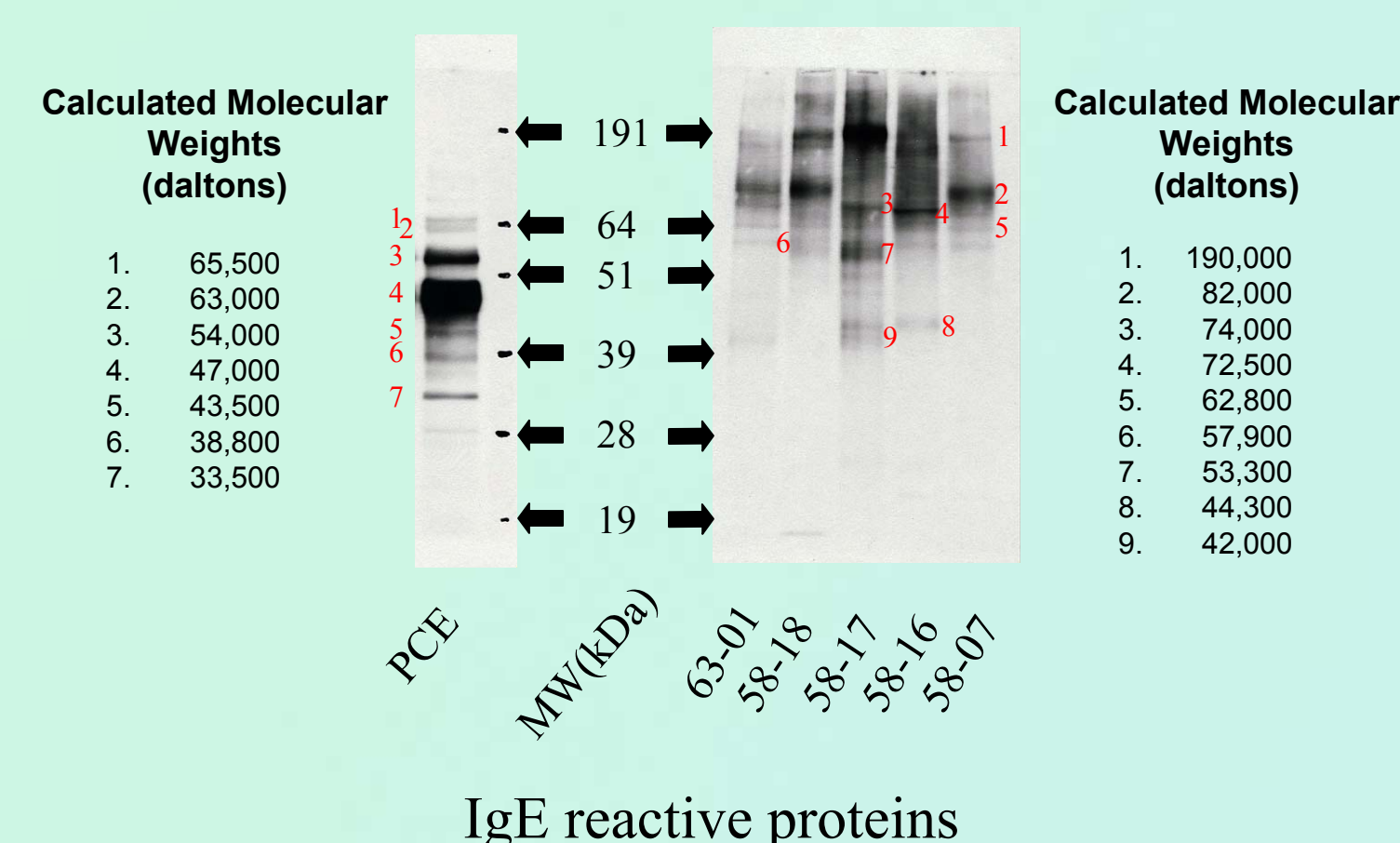
- Mice were anaesthetized by inhalation of a mixture of isoflurane/oxygen and given 4 doses of either 50 µl HBSS, 1, 10, 100 µg PCE (treatment), 3 doses of HBSS and one appropriate dose of PCE (inflammatory control) or 10 µg of *Metarhizium anisopliae* extract (MACA)/50 µl HBSS (positive control) by involuntary aspiration (IA) on the schedule described below.
- Mice were killed on day 0 (prior to the fourth IA exposure), day 1, and day 3 by an overdose of sodium pentobarbital and exsanguinated by cardiac puncture.
- Sera were collected and stored as described above.
- Lungs were lavaged with HBSS (BALF); total cell number was determined.
- Cytospin preparations were made by centrifuging BALF cells on to glass slides, and differential cell counts were performed.
- BALF were also analyzed for total protein and LDH using a Cobas Fara II centrifugal spectrophotometer (Hoffman-LaRoche, Branchburg, NJ).



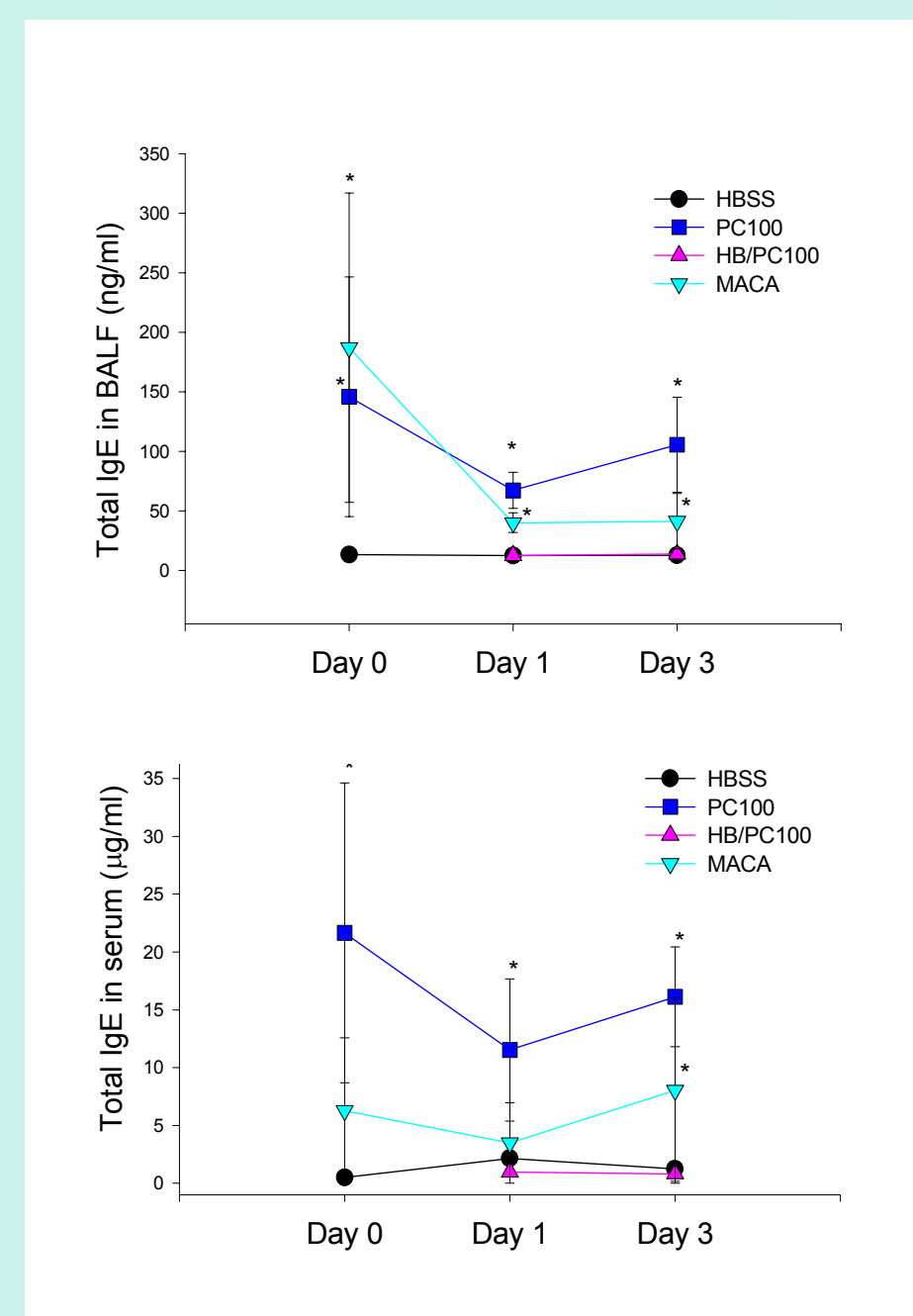
3. Cell culture

- The human alveolar epithelial A549 cell line was obtained from the American Type Culture Collection (Rockville, MD). Cells were maintained at 37 °C in a 5% CO₂ humidified incubator in Ham's F12K medium supplemented with 10% (v/v) fetal bovine serum (FBS; Gibco, Carlsbad, CA), 100 U/ml penicillin, and 100 µg/ml streptomycin (Gibco).
- Cells (2.5 x 10⁵ cells/ml) will be incubated in 12 well tissue culture plates (Fisher Scientific Co., Corning, NV) in a volume of 1 ml per well for 24 hr. Supernatant will be replaced with medium containing 0.1 to 10 µg/ml of chrysolysin provided by Dr. Steve Vesper (US EPA, Cincinnati, OH). Cells will be incubated for 6 and 24 hr and supernatant was subjected to ELISA for TNF-α, IL-6 and IL-8 analysis. Cytokine mRNA expression will be assayed by Ribonuclease protection assay.

Results



PCE100 and HB/PC100 cause pulmonary cell damage and edema



PCE(100 µg/ml) induces increases in total IgE compared to HBSS treated mice

Note: PCE (1 and 10 µg/ml) did not increase the level of total IgE.

Impacts

- This project provides not only the further assessment of a mouse model of allergic responses to indoor fungi, but also an assessment of the relative potency of IgE inducing proteins.
- This data will provide a better risk assessment to aid the Office of Air and the regional offices to appropriately inform the public on the hazards associated with moldy buildings.

Future Directions

- 2D gel and mass spectrometric analysis of IgE binding proteins from both SCE and PCE
- Western blot analysis of SCE using both human and mouse sera
- Additional *in vivo* experiments to determine an optimal experimental dose of PCE and to assess the respiratory physiological responses to this extract
- Assessment of the pro-inflammatory response to chrysolysin for potential adjuvancy in allergy induction
- Assessment of the relative potency of the IgE inducing proteins from MACA, SCE, and PCE compared to well characterized allergens such as house dust mite and alcalase

Conclusions

PCE (100 µg/ml) causes increases in immune cells in BALF compared to HBSS treated mice
Note: HB/PC100 (inflammatory control for PC100) induced significant influx of neutrophil

- PCE and SCE contain IgE-inducing proteins demonstrating a potential role in allergy induction.
- PCE may be less potent at inducing allergic responses than MACA.



SOLVING AGENCY PROBLEMS